IN THE UNITED STATES PATENT & TRADEMARK OFFICE

Re: Application
M.E. Ludgate
Serial No: 09/509,687
Filed: 19 April 2000

For: BIOASSAY FOR THYROID STIMULATING ANTIBODIES

DECLARATION UNDER RULE 1.132

I, Marian Elizabeth Ludgate, solemnly state and declare as follows.

That I am a citizen of Great Britain and resident of 6 Cyncoed Rise, Cardiff, CF23 6SF, Great Britain.

That I graduated from Cardiff University with a BSc in 1973.

That I hold a PhD in Tumour Immunology from the University of Wales College of Medicine, obtained in 1978.

That I have worked in the Department of Medicine (Endocrinology, Metabolism and Diabetes) at the University of Wales College of Medicine, since 1998, and currently hold the position of Senior Lecturer in said Department.

That I have been active in the field of disorders of the thyroid since 1982.

That I have read application 09/509,687 and the two documents cited against it, that is Sunthornthepvarakul et al., N.E.J.M, Vol 332, No. 3, pages 155-160 (D1), and Clifton-Bligh et al., Journal of Clinical Endocrinology and Metabolism, Vol. 82, No. 4, pages 1094-1100 (D2).

That I do not believe D1 and/or D2 disclose the invention in application 09/509,687, for the following reasons.

The terms 'transiently' and 'stably' refer to two different methods of transfecting cells, and the cells thus transfected are used for different purposes.

The provision of transiently transfected cells is achieved by the use of a plasmid with a reporter-gene construct. The construct will usually comprise an enhancer or promoter with a reporter gene downstream, such as the luciferase gene. Following transfection, the cells are harvested 48 to 72 hours later, while the cells are in the transient expression phase, and assayed for activity, for example luciferase activity.

Thus, in transient transfection a reporter gene is expressed prior to integration into a chromosome, and only a fraction of the templates are assembled into nucleosomal chromatin. Transient transfections provide a rapid means for identifying promoters

and enhancers, but as the foreign sequence(s) are not integrated into the host cell DNA, offspring of the host cell do not consistently inherit them. Accordingly, transient transfection is used to produce cells that can be used once, i.e. for a single generation, and thrown away.

The provision of a stably transfected cell line is achieved by the use of a plasmid which encodes for antibiotic resistance. This can either be a separate plasmid from that encoding the gene of interest, in this case the human TSHR, or a construct comprising both the antibiotic resistance and the gene of interest. Following transfection, the cells are selected using the antibiotic. In this way only those cells which have incorporated the plasmid(s) into their genome will be able to continue to grow.

'Stably' transfected cells therefore carry the test plasmid integrated into a chromosome, and hence the template DNA is in a normal chromatin structure. Stably transfected cells can reveal position effects, and because of the complete integration of the foreign sequence(s) into the cell DNA structure, they are passed on during cell division. Accordingly, stably transfected cells produce a cell line, which survives for many generations, and can be used at any time.

D1 and D2 both disclose **transiently** transfected cells, while 09/509,687 relates to **stably** transfected cells.

This is clear from page 156 of D1, which describes the construction of the expression vectors, and the "Functional Studies of the Thyrotropin Receptors in a Transient Transfection System". Paragraph 5 describes the structure of the reporter-gene construct (using luciferase), while paragraph 6 describes how the cells were transfected with this construct for 48 hours, and then assayed for luciferase activity.

Similarly, in the results section of D2, it states that JEG3 cells were 'transfected by a 5-h exposure to calcium phosphate containing αLUC [luciferase gene] reporter', and 40 hours later the cells were lysed and assayed for luciferase activity (page 1095, left column, paragraph 3 - right column, paragraph 1). The 'Results' section also goes on to say that 'expression vectors encoding wild-type or mutant receptor were transiently cotransfected with a reporter gene consisting of the glycoprotein hormone α-subunit promoter and luciferase gene (αLUC) into JEG3 cells' (page 1095 right column, last line and page 1096, left column, lines 1-4).

In contrast, 09/509,687 relates to 'stably transfected' (page 3, line 26, page 5, line 30, page 7, lines 14-23) cells expressing human TSH-R. This is apparent from the method used in Example 1, where JP09 cells are transfected with an 'expression vector carrying the puromycin resistance gene' (page 9, lines 25-26), and the successfully transfected cells are identified by exposing them to puromycin. The same method is used in Examples 2 and 4. Additionally, it can further be inferred from the use of these cells in a kit, which is re-usable, that they must be stably transfected.

In conclusion, the cells in D1 and D2 are transfected with plasmids that have reporter-gene constructs, meaning that they are transiently transfected, and accordingly are meant to be 'used once and thrown away'. The cells in 09/509,687

are transfected with plasmids encoding for antibiotic resistance, meaning that they are stably transfected, and are meant to be re-used.

For these reasons do D1 and D2 neither explicitly disclose, nor can it be inferred from them, that they relate to stably transfected cells as claimed in application 09/509,687. I do therefore not believe that D1 or D2 disclose the invention in application 09/509,687.

I further declare that all statements made herein of my own knowledge are true and that all statements made or information given are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, and that such wilful false statements may jeopardise the validity of an application or any patent issuing thereon.

M Ludgate

Marian Elizabeth Ludgate

Date: 10/5/4